Klebsiella pneumoniae ST147: and then there were three carbapenemases

L F Mataseje [1], L Chen [2 3], G Peirano [4 5], K Fakharuddin [1], B Kreiswith [2 3], M Mulvey [1], J D D Pitout [6 7 8,*]

[1] National Microbiology Laboratory, Winnipeg, MB, Canada.

[2] Center for Discovery and Innovation, Hackensack Meridian Health, Nutley, USA.

[3] Hackensack Meridian School of Medicine at, Seton Hall University, Nutley, NJ, USA.

[4] Division of Microbiology, Alberta Precision Laboratories, Calgary, AB, Canada.

[5] Cummings School of Medicine, University of Calgary, 3535 Research Road NW, Calgary, AB, #9T2L 2K8, Canada.

[6] Division of Microbiology, Alberta Precision Laboratories, Calgary, AB, Canada. jpitout@ucalgary.ca.

[7] Cummings School of Medicine, University of Calgary, 3535 Research Road NW, Calgary, AB, #9T2L 2K8, Canada. jpitout@ucalgary.ca.

[8] University of Pretoria, Pretoria, Gauteng, South Africa. jpitout@ucalgary.ca.

* Correspondence to J. D. D. Pitout. Email: jpitout@ucalgary.ca

Abstract

Gram-negative bacteria containing three different carbapenemases are extremely rare. Klebsiella pneumoniae (N22-925) with KPC-2, NDM-1, and OXA-48 was obtained from a Canadian patient with recent hospitalization in Romania. Short and long read whole genome sequencing showed that the bla_{KPC-2} was situated on a 214 kb IncFIB(K)/IncFII(K) plasmid, the bla_{NDM-1} on a 104 kb IncFIB (pQil)/IncFII(K) plasmid, and the bla_{OXA-48} on a 64 kb IncL plasmid. These plasmids were conjugated to Escherichia coli J53. N22-925 belonged to a unique ST147 cluster that is likely endemic in Romania. This case emphasizes the need for rapid carbapenemase screening in patients from endemic regions. We described the first complete genome sequence of a K. pneumoniae isolate with three different carbapenemases, providing a reference for future studies on this rarely reported occurrence.

Keywords: Bla KPC-2; Bla NDM-1; Bla OXA-48; K. pneumoniae ST147.

Introduction

The World Health Organization identified carbapenem-resistant Enterobacterales as critical-priority bacteria [1]. Carbapenemases are important causes of carbapenem resistance because they inactivate carbapenems. Furthermore, carbapenemase genes can be transferred between Enterobacterales species [2]. The most common carbapenemases among Enterobacterales are

KPCs, IMPs, VIMs, NDMs, and OXA-48-like enzymes [2]. *Klebsiella pneumoniae* is the most common carbapenemase-producing Enterobacterales species [3, 4]. Gram-negative bacteria containing three different carbapenemases are extremely rare [3,4,5].

Overseas travel is as a risk factor for the acquisition of infections due to antimicrobial resistant organisms [6]. Out-of-country hospitalization is an important risk factor for colonization or infection with carbapenemase producing organisms [7,8,9] and was responsible for mortality due to nosocomial transmission [10]. It is essential to rapidly identify patients colonized or infected by these bacteria and place them on appropriate infection control precautions [10].

A *K. pneumoniae* isolate (N22-925) that tested positive for NDM, OXA-48-like, and KPC was obtained from a patient admitted to a Canadian health care facility. The patient was recently hospitalized in Romania. The patient was immediately placed on contact precautions. No secondary spread has been documented during his stay. Long and short read whole genome sequencing was used to characterize the isolate.

Materials and methods

Patient and isolate

During March 2022, a middle-aged male with suspected pulmonary embolus was admitted to a Calgary hospital. He recently admitted to a medical center in Romania for a hypertensive crisis. He did not receive antibiotics during his stay. Routine screening is done in Calgary on admission for patients who received hemodialysis or have been hospitalized for > 24 h outside of Canada within the past 6 months [11]. Routine admission screening using rectal swabs was done for antimicrobial resistant organisms, including carbapenem-resistant Gram-negative organisms. Growth of a Gram-negative bacterium was obtained on CHROMID® CARBA SMART Agar (bioMérieux Canada, Saint-Laurent, Quebec) and identified as *K. pneumoniae* using matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF/MS) (Vitek AMS; bioMérieux Vitek Systems Inc., Hazelwood, MO). Carbapenemase testing with NG-Test CARBA 5® [12] was positive for KPC, NDM, and OXA-48-like enzymes. PCR for carbapenemase genes was positive for *bla*$_{\text{KPC}}$, *bla*$_{\text{NDM}}$, and *bla*$_{\text{OXA-48-like}}$ [13]. The isolate (N22-925) was referred to the National Microbiology Laboratory, Manitoba, Canada, for additional characterization (long and short read sequencing).

Antimicrobial susceptibilities

Minimum inhibitory concentrations (MICs) were determined using the Thermo Scientific™ Sensititre™ Gram-negative GN6F AST Plate (Thermo Scientific, Winnipeg, Manitoba, Canada) and interpreted by using CLSI guidelines for broth dilution [14]. Cefiderocol MICs were determined using *E*-test (bioMerieux Inc., Hazelwood, MO, USA) according to the manufacturer's instructions**.**

Whole genome sequencing

Total DNA was extracted from pure culture using Qiagen DNeasy Plate kits (Qiagen, Toronto, Canada). Illumina libraries were created with Nextera XT kits (Illumina, San Diego, USA) where paired end 150 bp indexed reads were generated on an Illumina NextSeq™

platform using the 500/550 v2.5 (300 cycles) preparation kits. MinION (Nanopore Technologies, Oxford, UK) sequencing libraries were prepped using the one-dimensional rapid kit (SQK-RBK 004) and R9.4 flowcells and run on MinKNOW v5.0.0. De novo hybrid assemblies were done using Unicycler 0.4.7 [15]. Assembled sequence data was analyzed for Multi Locus Sequence Typing (MLST), resistance genes, and plasmid typing using StarAMR tool (https://github.com/phac-nml/staramr).

Conjugation/transformation

Experiments were performed by mating-out assays with nutrient agar containing meropenem (0.125 µg/mL) and using *Escherichia coli* J53 (azide 100 µg/mL or rifampicin 150 µg/mL) as recipient. Frequency of transfer was calculated as the number of transconjugants per the number of donors as described before [16]. Carbapenemase plasmids were also transformed into *E. coli* ElectroMax™ DH10B cells (Invitrogen, Carlsbad, CA, USA) and selected on 0.125 mg/L meropenem.

Results and discussion

The clinical laboratory acts as an early warning system, alerting the medical community to new resistance mechanisms present in bacteria [17]. In our study, NG-Test CARBA 5® assay was performed on bacterial colonies obtained from CHROMID® CARBA SMART Agar [12]. Results were available within 10 min that correlated with our in-house PCR [13] and subsequent WGS analysis.

The *K. pneumoniae* isolate (N22-925) was identified as ST147 and contained nine plasmids (ranging from 2 to 214 kb in sizes) and was positive for the following antimicrobial resistance (AMR) determinants (i.e., *bla*$_{\text{KPC-2}}$, *bla*$_{\text{NDM-1}}$, *bla*$_{\text{OXA-48}}$, *bla*$_{\text{CTX-M-15}}$ (three copies), *bla*$_{\text{SHV-11}}$, *bla*$_{\text{OXA-9}}$, *bla*$_{\text{TEM-1}}$, *fosA*, *oqxA*, *oqxB*, *catA1*, *aph(3')-VI*, *qnrS1*, and *qnrB19*). *K. pneumoniae* ST147 is an emerging global multidrug-resistant (MDR) high-risk clone that is linked with various carbapenemases (i.e., KPCs, NDMs, OXA-48-like, and VIMs) [18]. ST147 is endemic in India, Europe (especially Italy, Greece), and North African countries (i.e., Algeria, Tunisia, Libya) and has been introduced into non-endemic regions (i.e., North America), leading to nosocomial outbreaks [18]. ST147 with OXA-48 was previously reported from wastewater in Romania [19]. High-risk multidrug-resistant clones are important sources for horizontal and vertical transmission of antimicrobial resistance determinants [20].

The chromosomal/plasmid locations of the different AMR determinants are shown in Table 1. Parsnp [21] core genome phylogenetic analysis of N22-925 and 526 other ST147 *K. pneumoniae* genomes from NCBI RefSeq database (dated as Aug 1, 2022) showed that N22-925 is located at a small branch of three strains containing the capsule synthesis locus (KL) 112, and is separate from ST147 with KL64 (Fig. 1). The other two ST147 KL112 strains were KP51483 isolated from Czech Republic in 2019 (biosample accession no. SAMN16933290) and EuSCAPE_RO108 from Romania in 2013 (biosample accession no. SAMEA3673106). Recombination free core snp analysis using snippy v4.6 (https://github.com/tseemann/snippy) showed that N22-925 is different from KP51483 and EuSCAPE_RO108 with 21 and 93 core snps, respectively.

Table 1. Chromosomal and plasmid locations of the different antimicrobial resistance determinants in *K. pneumoniae* ST147

Location	Replicon	Size (kb)	Antimicrobial resistance determinant
Chromosome	NA	5286	$bla_{CTX\text{-}M\text{-}15}$ (2 copies), $bla_{SHV\text{-}11}$, *fosA*, *oqxA*, *oqxB*
pKPC_N22-925	IncFIB(K) IncFII(K)	214	$bla_{KPC\text{-}2}$, $bla_{OXA\text{-}9}$, $bla_{TEM\text{-}1}$, *catA1*
pNDM_N22-925	IncFIB(pQil) IncF(K)	104	$bla_{NDM\text{-}1}$, $bla_{CTX\text{-}M\text{-}15}$, *aph(3')-VI*, *qnrS1*
pOXA-48_N22-925	IncL	64	$bla_{OXA\text{-}48}$
pQnrB19_N22-925	Col440I	6	*qnrB19*

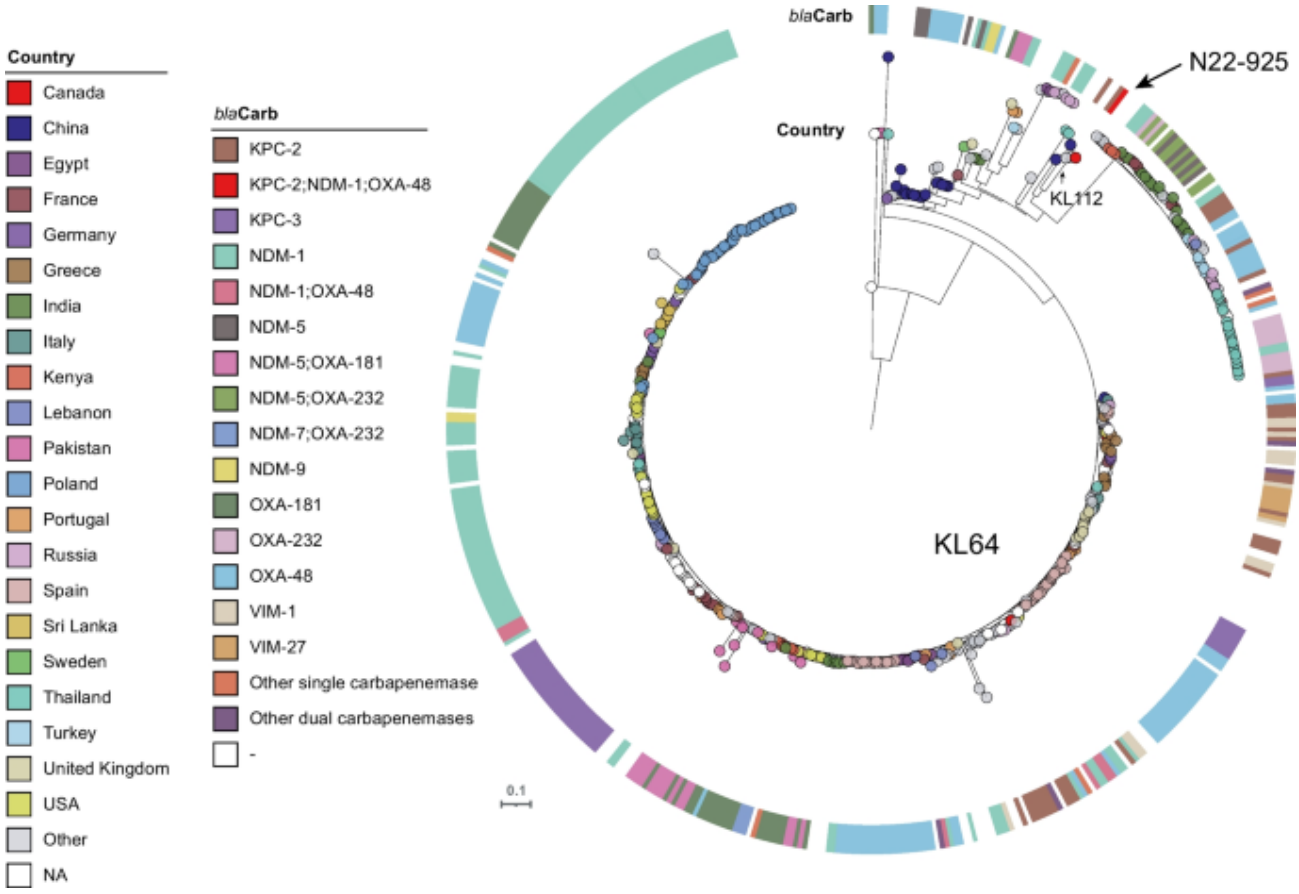

Fig. 1. Phylogenetic analysis of N22-925 and publicly available genomes of *K. pneumoniae* ST147 ($n = 526$)

K. pneumoniae isolate (N22-925) tested resistant to (MICs in g/L): piperacillin/tazobactam (> 64/4), ceftazidime/avibactam (> 16/4), ceftolozone/tazobactam > 16/4), imipenem/relebactam (> 8/4), meropenem/vaborbactam (> 16/8), ceftriaxone (> 64), ceftazidime (> 64), cefepime (> 64), aztreonam (> 32), meropenem (> 16), ertapenem (> 8), ciprofloxacin (> 2), levofloxacin (> 4), and colistin (> 8). The isolate was intermediate for doxycycline (8) and minocycline (8) and sensitive to tigecycline (1), amikacin (4), gentamicin (2), tobramycin (2), plazomicin (0.5), cefiderocol (4), and trimethoprim-sulfamethoxazole (2/38).

The *bla*KPC-2 was situated on a 214 kb IncFIB(K)/IncFII(K) plasmid (pKPC_N22-925) (Fig. 2A) that was similar (with > 99% blast query coverage and > 99% nucleotide identities) to plasmid p51483_KPC from Czech Republic (in the strain KP51483) (JAFIQI010000011) and pC608_1 from the USA (CP067576.1).

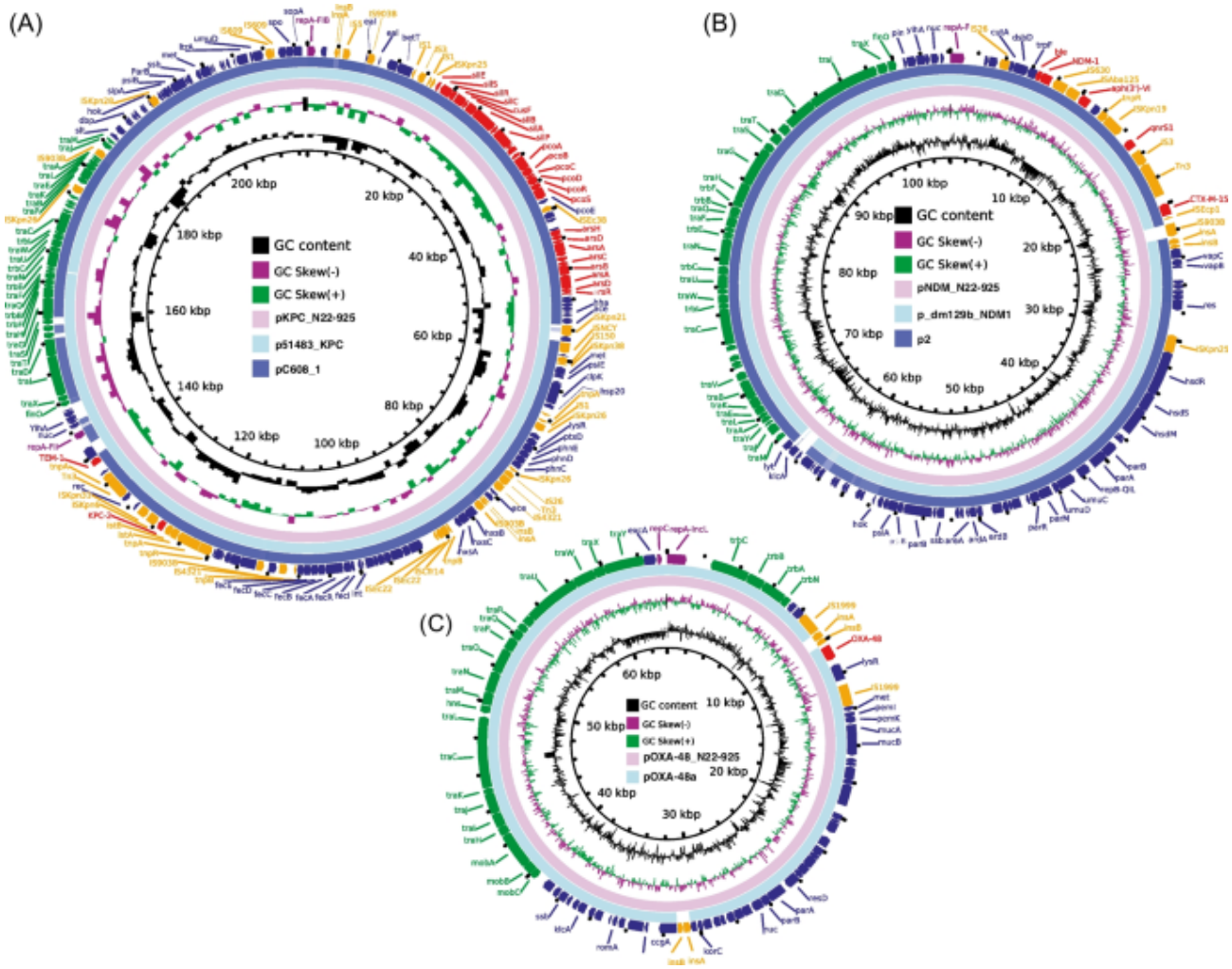

Fig. 2. N22-925 plasmids with carbapenemases: pKPC_N22-925 (**A**), pNDM_N22-925 (**B**), pOXA-48_N22-925 (**C**)

The *bla*NDM-1 was situated on 104 kb IncFIB (pQil)/IncFII (K) plasmid (pNDM_N22-925) (Fig. 2B) that was similar (with > 97% blast query coverage and > 99% nucleotide identities) to p_dm129b_NDM1 from Bangladesh (CP095596) and plasmid p2 (CP009115, [22]) from the USA. Despite being classified as the same IncFIIK2 plasmid by PlasmidFinder [23], pNDM_N22-925 only showed ~ 96.5% nucleotide identities and ~ 70% query coverage against the pKpQIL or pKpQIL-IT plasmids (Fig. 3). The region between *vagD* and *ardA* showed > 99.5% identities between pNDM_N22-925 and pKpQIL or pKpQIL-IT, and only ~ 90% nucleotide identities in the region between *ardA* and *traI*, and ~ 98% identities in the region between *traT* and *repA2*. The most significant difference between pNDM_N22-925 and pKpQIL or pKpQIL-IT is at the IncFII replication gene and antimicrobial resistance (AMR) loci. pKpQIL and pKpQIL-IT harbor the pKPN-like IncFII replication genes, while pNDM_N22-925 and p_dm129b_NDM1 carry the pKDO1-like IncFII replication gene [24].

The two IncFII (pKPN-like and pKDO1-like) replication genes only had ~ 40% nucleotide sequence identities. In addition, the four plasmids carry different resistance genes in the AMR loci. pKpQIL and pKpQIL-IT harbor *bla*$_{KPC-3}$ (in Tn4401a) and *bla*$_{TEM-1}$, while pNDM_N22-925 and p_dm129b_NDM1 carry *bla*$_{NDM-1}$ (in ΔTn125), *aph(3')-VI*, *qnrS1*, and *bla*$_{CTX-M-15}$. The sequence analysis indicated that the *bla*$_{NDM}$-harboring IncFIB (pQil)/IncFII (K) plasmids (e.g., pNDM_N22-925) and the *bla*$_{KPC}$-harboring IncFIB (pQil)/IncFII (K) plasmids were not closely related, and it is possible that the *bla*$_{NDM}$-harboring IncFIB (pQil)/IncFII (K) plasmids have evolved through multiple recombination events from different plasmid backbones and the acquisitions of different AMR genes.

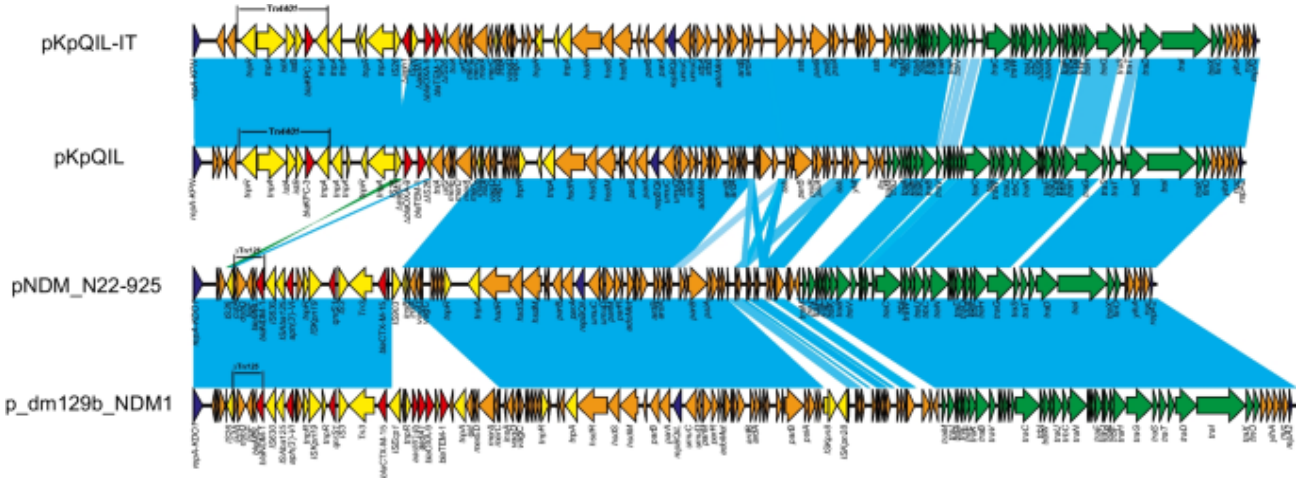

Fig. 3. The plasmid structures of pKpQIL, pKpQIL-IT, pNDM_N22-925, and p_dm129b_NDM1. Light blue shading denotes shared regions of homology with > 90% identities. ORFs are portrayed by arrows and colored based on predicted gene function. Orange arrows indicate plasmid scaffold regions. The genes associated with the tra locus are indicated by green arrows, and replication associated genes are denoted as dark blue arrows. Antimicrobial resistance genes are indicated by red arrows, while the accessory genes are indicated by yellow arrows

The *bla*$_{OXA-48}$ was situated on a 64 kb IncL plasmid (pOXA-48_N22-925) (Fig. 2C) that was similar (with > 97% blast query coverage and > 99% nucleotide identities) to global OXA-48 InL plasmids [25]. Conjugation experiment showed that plasmids pOXA-48_N22-925, pNDM_N22-925, and pKPC_N22-925 were able to conjugate to *E. coli* J53. Transfer frequencies were as follows: pOXA-48_N22-925 (1×10^{-4}), pNDM_N22-925 (1×10^{-4}), and pKPC_N22-925 (1×10^{-5}). Transformation of all three carbapenemase plasmids into *E. coli* DH10B was also successful.

Gram-negative bacteria containing three different carbapenemases are extremely rare. The high-risk clone *K. pneumoniae* ST147 from this study contained nine plasmids and was considered as a potential source for the horizontal spread of several carbapenemases. A ST147 cluster containing KL112 is likely endemic in Romania.

This case emphasizes the need for vigilant screening for carbapenem-resistant Gram-negative bacteria in patients with a recent history of travel to endemic areas using methodologies that can rapidly identify the types of carbapenemases. We described here the first complete genome sequence of a *K. pneumoniae* isolate with three different carbapenemases, providing a reference for future studies on this rarely reported occurrence.

Data availability

Sequence data was uploaded to NCBI (BioProject PRJNA861987).

Code availability

Sequence data is available at NCBI (BioProject PRJNA861987).

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Contributions

All authors designed the study and approved the manuscript. LM, LC, KF, and GP performed PCR, WGS, bioinformatics, conjugation, and transformation studies. JP, LM, LC, and GP combined the clinical and genomic data. JP wrote the 1st draft of the manuscript.

Ethics declarations

Ethics approval

Ethics approval for this study was obtained through the University of Calgary Conjoint Health Research Ethics Board (REB17-1010).

Consent to participate

Not applicable.

Consent for publication

This manuscript has not been published and is not being considered for publication elsewhere.

Conflict of interest

The authors declare no competing interests.

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